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HI6 as an antidote to soman poisoning in rhesus monkey respiratory muscles in-vitro

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This study was carried out to evaluate the rhesus monkey as a model for man with respect to oxime-induced acetylcholinesterase reactivation as a mechanism contributing to restoration of soman poisoned neuromuscular function. In-vitro neuromuscular blockade in intercostal and diaphragm muscle strips of rhesus monkeys was induced by exposing them for 2.5 or 15 min to the cholinesterase inhibitor soman. Subsequent treatment with the oxime HI6 produced only a partial reversal of this blockade. Only a minor part of the recovery obtained could be attributed to enzyme reactivation and suggested that this species responded in a manner closer to that reported in man than other animal species studied.

The bispyridinium oxime HI6 (Schoene 1967) combined with a cholinolytic has improved the effectiveness of the treatment of soman poisoning in several animal species (Bošković & Stern 1970; Oldiges & Schoene 1970; Schenk et al 1976; Wolthuis et al 1976). These results prompted further studies into the mechanism of therapeutic action of this oxime since poisoning by the acetylcholinesterase inhibitor soman is considered to be resistant to oxime treatment due to rapid 'ageing' of the enzyme-inhibitor complex to a form resistant to reactivation by oximes (Fleisher & Harris 1965).

Wolthuis et al (1981) reported that HI6 reversed soman induced neuromuscular blockade in respiratory muscles of rats, guinea-pigs and beagles in-vitro. Analysis of the recovery indicated that the return of neuromuscular function was due, in part, to oxime-induced reactivation of unaged soman inhibited acetylcholinesterase. In contrast, HI6 did not reverse soman-induced neuromuscular paralysis of human intercostal muscle in-vitro, although effective restoration of function was obtained following poisoning by the 'oxime sensitive' acetylcholinesterase inhibitor sarin. Smith et al (1981) confirmed these findings and suggested that fundamental differences existed between neuromuscular junctions of man and the other animal species studied since, in addition to the apparent absence of acetylcholinesterase reactivation, direct oxime actions found to contribute to neuromuscular recovery in rodent respiratory muscles were also absent in man. The objective of this study was to evaluate the rhesus monkey as a model for man with respect to oxime-induced acetylcholinesterase reactivation as a mechanism contributing to restoration of soman poisoned neuromuscular function. The action of HI6 on soman poisoned respiratory muscles of the rhesus monkey has been investigated in-vitro.

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MATERIALS AND METHODS

Drugs and chemicals

Soman (*O*-pinacolyl-methylphosphonofluoridate) was synthesized at the Chemical Defence Establishment at Porton Down, England. The oxime HI6 1-(2-hydroxyiminoethylpyridinium)-1-(4-carboxamidopyridinium) dimethyl ether was synthesized at the Chemical Research Department of the Prins Maurits Laboratory TNO, Holland. Ketamine hydrochloride, halothane and (+)-tubocurarine chloride were purchased from Parke-Davis Ltd, May and Baker Ltd and Koch-Light Laboratories Ltd, respectively.

Preparation of muscle strips

Rhesus monkeys (5–9 kg) of either sex were initially anaesthetized with ketamine hydrochloride (10 mg kg^{-1} im), tracheotomized and then anaesthesia maintained with 0·5 to 2·0% halothane, generated using a mark 2 Fluotech (Cyprane Ltd), in two parts N_2O to one part O_2 passed to the animal via the tracheal cannula.

Intercostal muscle biopsies were removed in the axillary line from the lower third of the rib cage. The surgery produced pneumothorax which required the animal to be maintained under anaesthesia using a positive pressure ventilator (Nuffield Anaesthesia Ventilator Series 200). This technique allowed a further set of biopsies (both intercostal and diaphragm muscle) to be removed on completion of experiments with the first series of tissues. During

the delay between removal of the first and second set of biopsies depth of anaesthesia and viability of the animal were ensured by monitoring blood pressure via a cannula implanted into the right carotid artery and connected to a physiological pressure transducer (Bell & Howell) and direct writing pen recorder (Ormed Engineering Ltd). Animals were maintained under anaesthesia without cardiovascular deterioration for more than 6 h. Following removal of the second set of muscle biopsies the monkeys were killed by intravenous administration of an overdose of sodium pentobarbitone.

External intercostal muscles were prepared for stimulation using the technique described for human muscle by Wolthuis et al (1981). For studies of the diaphragm muscle, a 5 mm wide strip was cut from the hemidiaphragm from the point of rib attachment to the apex, parallel to the direction of the muscle fibres. The strip was ligated at positions above the level of entry of the phrenic nerve to the muscle and below the motor endplates producing a preparation of 40 mm in length. The tissue was mounted in a manner similar to that described for the intercostal muscle.

Both diaphragm and intercostal muscle tissues were suspended in Krebs-Ringer bicarbonate at 37 °C and gassed with 95% O_2 and 5% CO_2. The tissues were stimulated (pulse width 3–4 μs, pulse current 600–1100 mA) via bipolar platinum electrodes using a field stimulation technique (Wolthuis et al 1981). Muscle contractions were recorded using an Ormed type 4151 isometric force transducer and Ormed direct writing pen recorder.

Experimental protocols

Single twitch responses (0·1 Hz) were recorded throughout the experiments unless otherwise stated. Tetanus responses were measured at 25, 50, 100 and 200 Hz stimulation frequencies, each for 3 s with a resting time of 30 s between tetani. The experimental protocol is represented schematically in Fig. 1. Following control tetani (Test A), soman (1×10^{-6} M) was added to the tissue bath and left in contact with the tissue for 2·5 or 15 min. After washout of soman, tetanic function was measured (Test B) after which HI6 ($1{\cdot}5 \times 10^{-3}$ M) was added to the bath. Fifteen minutes later, following removal of the oxime by washing, neuromuscular function

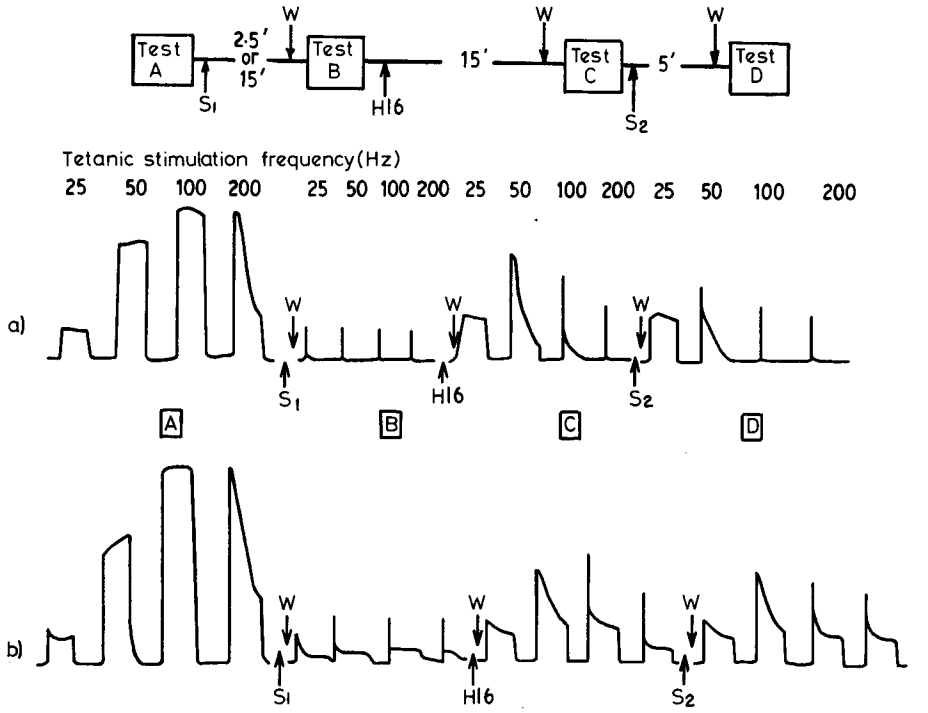

FIG. 1. Experimental (traced) records of the effect of HI6 on neuromuscular blockade produced by (a) 2·5 min exposure to soman and, (b) 15 min exposure to soman in rhesus monkey intercostal muscle preparations. A–D: tests of neuromuscular function represented schematically, S_1: soman ($1{\cdot}1 \times 10^{-6}$ M), H16: ($1{\cdot}5 \times 10^{-3}$ M), W: Washout, S_2: soman ($2{\cdot}2 \times 10^{-6}$ M).

was retested (Test C) and a second dose of soman (2×10^{-6} M) added to the bath. Tetanic function was tested 5 min later following washout of soman.

Neuromuscular recovery following soman poisoning was calculated by measuring the height of tetanic contraction at the mid point (i.e. 1·5 s after onset of stimulation) and expressed as a percentage of the control height. Changes in tetanic heights from tests B to C and C to D were analysed statistically using the Kolmogorov Smirnov two sample test (Campbell 1974).

RESULTS AND DISCUSSION

HI6 partially restored neuromuscular function in rhesus monkey respiratory muscle preparations following 2·5 or 15 min exposure to soman (Table 1, Fig. 1). After 2·5 min exposure to soman total recovery of neuromuscular function was observed in intercostal muscle stimulated at 25, partial recovery at 50, but no recovery at 100 and 200 Hz. The response to 50 Hz stimulation was significantly ($P < 0·05$) reduced following addition of the second dose of soman suggesting that acetylcholinesterase reactivation was responsible, at least in part, for the restoration of neuromuscular function. A proportion of the recovery observed with both stimulation frequencies was resistant to reinhibition by the second dose of soman indicating that at least one mechanism not related to acetylcholinesterase reactivation also contributed to the observed recovery. Since HI6 had been removed from the tissue bath before the addition of the second dose of soman it is unlikely that direct oxime actions (Smith & Muir 1977; Smith et al 1981) could be responsible for this recovery. It is possible that mechanisms unrelated to oxime action, e.g. adaptation of the endplates to the prolonged presence of high levels of acetylcholine (Meeter & Wolthius 1968; Meeter 1969) may be responsible for this recovery.

Only two diaphragm preparations were used thus statistical analysis of the results was not made. The overall degree of recovery obtained was lower than that described for the intercostal muscle but changes induced by addition of the second doses of soman were similar (Table 1c).

A slightly greater degree of neuromuscular recovery was observed in intercostal muscle exposed to soman for 15 min before oxime administration (Table 1b). No significant changes in the degree of recovery were observed following the second dose of soman suggesting that acetylcholinesterase reactivation did not contribute to the recovery. It has been suggested (Wolthuis et al 1981) that 15 min exposure to soman in isolated rat intercostal muscle preparations produced non-specific binding of soman to rib and tendon attachments. This bound soman was not removed by the washout process and reinhibited oxime–regenerated acetylcholinesterase, obscuring any neuromuscular recovery observed as a product of this process. It would seem unlikely that this process could account for the results obtained in the present study since rhesus intercostal muscle preparations, unlike those of the rat, consist purely of a bundle of external intercostal muscle fibres. The resistance of the neuromuscular recovery to the second dose of soman, together with the fact that it was observed following washout of HI6 from the tissue bath suggests that it is due to a mechanism other than direct oxime action or oxime-induced reactivation of acetylcholinesterase.

The results obtained following 2·5 min exposure of the tissues to soman in this study are at variance with those reported in human muscle by Wolthuis et al (1981) where HI6-induced reactivation of acetylcholinesterase did not appear to contribute to neuromuscular recovery following soman poisoning. Owing to the limited number of rhesus monkey biopsies available during the study a full comparison could not be made between the response of the two

Table 1. Neuromuscular recovery following HI6 therapy in rhesus monkey (a and b) intercostal and (c) diaphragm muscle preparations at tests B, C and D in the experimental protocol represented schematically in Fig. 1. Table (a) and (c): recovery obtained following 2·5 min exposure to soman, Table 1(b): recovery obtained following 15 min exposure to soman. Tetanus heights are expressed as a mean percentage of control heights (±s.e.m.) for intercostal muscles (n = 5). Mean heights only are given for diaphragm results (n = 2). Hz: frequency of tetanic stimulation, Sig: Significance of changes of tetanic height between tests (NS: non-significant, * Significant, $P < 0·05$).

Hz	Test B	Sig B–C	Test C	Sig C–D	Test D
(a)					
25	4·0 ± 2·6	*	123·2 ± 17·5	NS	105·2 ± 12·7
50	3·9 ± 3·9	*	34·6 ± 5·7	*	12·1 ± 5·8
100	0	NS	0·9 ± 0·6	NS	0
200	0	NS	0	NS	0
(b)					
25	1·8 ± 1·8	*	74·3 ± 9·4	NS	82·5 ± 14·8
50	4·0 ± 2·5	*	43·7 ± 7·9	NS	18·0 ± 7·5
100	3·0 ± 2·2	*	13·1 ± 4·4	NS	8·2 ± 3·3
200	2·7 ± 1·9	NS	7·1 ± 3·0	NS	5·1 ± 3·9
(c)					
25	0		68·1		23·6
50	0		18·0		8·8
100	0		7·9		7·9
200	0		6·1		6·1

species to HI6 therapy. It has been suggested (Smith et al 1981) that fundamental differences may exist between the neuromuscular junction of human and rodent respiratory muscles which may account for the differing responses of these species to oxime therapy. These differences have been characterized by an absence of beneficial direct oxime actions in human muscle, present in both rats and guinea-pigs and a time to peak tension of twitch contraction eight times longer in the human muscle. In the present study direct oxime actions were not investigated in rhesus monkey muscle, but time to peak tension of twitch contraction was measured at 80 ms, faster than human muscle by a factor of 2.

In conclusion HI6 is partially effective in reversing soman-induced neuromuscular blockade of rhesus monkey respiratory muscle preparations in vitro but the recovery obtained is only to a small degree due to reactivation of soman-inhibited acetylcholinesterase. In this respect the rhesus monkey as an experimental model is closer to man than other species tested. From the studies reported by Wolthuis et al (1981), Smith et al (1981) and this study there is a species difference in the contribution of HI6 induced enzyme reactivation to recovery of soman poisoned neuromuscular function in the order dog > rat > guinea-pig, rhesus monkey > man. The mechanisms responsible for the differences in species response remain unclear.

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REFERENCES

Bošković, B., Stern, P. (1970) Arch. Toxikol. 26: 306

Campbell, R. C. (1974) Statistics for Biologists, Second Edition, Cambridge University Press

Fleisher, J. H., Harris, L. W. (1965) Biochem. Pharmacol. 14: 641

Meeter, E., Wolthuis, O. L. (1968) Eur. J. Pharmacol. 2: 377

Meeter, E. (1969) Acta Physiol. Pharmacol. Neerl 15: 243

Oldiges, H., Schoene, K. (1970) Arch. Toxicol. 26: 293

Schenk, J., Loffler, W., Weger, N. (1976) Toxicology 36: 71

Schoene, K. (1967) Darstellung und untersuching neuer acetylcholinesterase reaktivatoren, Thesis, Rota-Druk Buchbinderei, J. Krause, Freiburg

Smith, A. P., Muir, A. W. (1977) J. Pharm. Pharmacol. 29: 762

Smith, A. P., Van der Weil, H. J., Wolthuis, O. L. (1981) Eur. J. Pharmacol. 70: 371

Wolthuis, O. L., Clason-Van der Wiel, H. J., Visser, R. P. L. S. (1976) Ibid. 39: 417

Wolthuis, O. L., Vanwersch, R. A. P., Van der Wiel, H. J. (1981) Ibid. 70: 355.